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Long-read Sequencing in Inherited Retinal Dystrophies: A Systematic Review

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Topic: Inherited retinal dystrophies (IRDs) encompass a group of phenotypically and genetically heterogeneous disorders leading to progressive visual impairment. The advent of next-generation sequencing (NGS) has substantially improved genetic diagnosis rates; however, approximately 30% of IRD cases remain genetically unsolved ("missing heritability"). Long-read sequencing (LRS) has emerged as a complementary approach for identifying complex, inaccessible variants, including structural and deep intronic variants. This study systematically reviews the application of LRS in IRD genetics with descriptive synthesis.

Clinical Relevance: Inherited retinal dystrophies collectively affect approximately 1 in 3000 individuals and are a leading cause of inherited blindness worldwide. Accurate molecular diagnosis informs prognosis, genetic counseling, and eligibility for emerging gene therapies. While NGS represents the current standard diagnosis, its inability to capture certain variant types limits its diagnostic sensitivity. Integrating LRS could bridge this diagnostic gap and enhance personalized management.

Methods: A systematic search on PubMed (September 1, 2025) identified 26 studies investigating the use of LRS in patients with IRD (published between 2018 and September 1, 2025). Studies were eligible if they applied LRS for the genetic diagnosis of IRDs in humans, were available in full-text English, and reported IRD variants. Data extraction and synthesis followed Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines. Methodological quality was assessed using Murad's tool for case reports and case series, the Joanna Briggs Institute Checklist for Case Series, and the Quality Assessment of Diagnostic Accuracy Studies-2 tool. The review was registered prospectively on the International Prospective Register of Systematic Reviews (identifier, CRD42024602173).

Results: Our analysis identified 88 IRD variants described by LRS. Of these, 31% were structural, 31% single nucleotide, 19% splice site, 11% insertions/deletions, and 8% deep intronic. Long-read sequencing expanded the IRD genetic landscape by identifying or refining pathogenic variants in 34 genes across 81 patients. Studies also validated LRS for phasing and resolving technically challenging regions.

Conclusions: Long-read sequencing overcomes key limitations of short-read sequencing in the genetic diagnosis of IRDs. Although existing studies show high analytical rigor and increased diagnostic yield, most lack formal diagnostic accuracy metrics. Current evidence supports the potential incorporation of LRS into clinical workflows, with further prospective studies needed to define diagnostic accuracy, clinical utility, and implementation standards.

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Inherited retinal dystrophies (IRDs) are a heterogeneous group of genetic disorders characterized by progressive retinal degeneration, leading to visual impairment and eventual blindness.[1] These diseases result from dysfunction in photoreceptors or retinal pigment epithelium.[2] Inherited retinal dystrophies affect approximately 1 in 3000–4000 individuals worldwide and are classified into syndromic and nonsyndromic types.[1] Syndromic IRDs, such as Usher syndrome and Bardet–Biedl syndrome (BBS), involve visual impairment along with other systemic symptoms including hearing loss or kidney dysfunction.[3] Nonsyndromic IRDs, on the other hand, are confined to the eyes and include conditions such as retinitis pigmentosa (RP), the most common form which leads to night blindness and peripheral vision loss.[4] These diseases exhibit significant genetic and clinical heterogeneity, with overlapping phenotypes caused by variants in >300 genes (https://sph.uth.edu/retnet/) affecting pathways critical to vision, including phototransduction, the visual cycle, photoreceptor development, cellular respiration, and retinal homeostasis.[2]

Next-generation sequencing (NGS) technologies, also known as short-read sequencing (SRS) methods, revolutionized the research on IRDs by enabling rapid and cost-effective detection of genetic variants since its introduction in 2005.[5,6] Four years later, the first study utilizing NGS in RP research was published.[7] Next-generation sequencing led to the discovery of numerous novel IRD-associated genes, such as *KIZ*, *C21orf2*, *EMC1*, *KIAA1549*, *GPR125*, *ACBD5*, *DTHD1*, *and UBAP1L*.[8–10]

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Through targeted panels, whole-exome sequencing (WES), and whole-genome sequencing (WGS), NGS became pivotal in molecular diagnosis of IRDs, achieving high diagnostic yields at relatively low cost.[1] Nonetheless, targeted sequencing, or WES, left many cases unsolved, which urged WGS to resolve cases to identify novel and deep intronic variants.[11]

However, despite these advances, about 30% of RP cases remained unsolved. The main limitations of NGS arise from its short-read lengths and guanine-cytosine content bias,[12] which hinder the detection of large structural variants (SVs), repetitive regions (e.g., the *RPGR*-ORF15), and deep intronic variants. These gaps contribute to the so-called "missing heritability" in IRDs, underscoring the need for more comprehensive sequencing approaches such as long-read sequencing (LRS).

Long-read sequencing, also known as third-generation sequencing, was commercially introduced in the early 2010s, with Pacific Biosciences (PacBio) launching its technology in 2011 and Oxford Nanopore Technologies (ONT) following in 2015.[13] Detailed comparisons between ONT and PacBio platforms, including their respective strengths and limitations, have been comprehensively reviewed elsewhere.[5,14,15] Long-read sequencing minimizes polymerase chain reaction (PCR)–related biases by enabling direct sequencing of DNA.[16] Unlike SRS, it captures long stretches of DNA in a single read, enabling accurate reconstruction of complex genomic regions and facilitating the detection of SVs and defects in hardly accessible genomic regions by providing sufficient context to call and resolve variants regardless of their sequence composition.[17,18] Emerging applications further expand its utility by enabling high resolution detection of epigenetic markers.[19] Compared with traditional SRS, which usually require weeks for library construction, sequencing, and analysis, LRS offers a significantly faster turnaround of only a few days.[20] Despite its emerging utility, LRS remains expensive in terms of instruments and supplies, with relatively low throughput compared with SRS.[14,21] Its implementation can also be constrained by the need for standardized pipelines, robust benchmarking, substantial computational infrastructure, and specialized bioinformatic expertise.[14,22]

Despite the major advances achieved through NGS, approximately 30% of IRD cases remain genetically unexplained. Long-read sequencing offers a complementary approach to close this diagnostic gap by detecting previously inaccessible variants and improving variant interpretation. Therefore, the current study systematically reviews the application of LRS to decipher the genetics of IRDs.

The objectives of this review are to:

- Review the quality of the included studies using design-specific quality assessment tools (Murad's tool for methodological quality and synthesis of case series and case reports, the Joanna Briggs Institute [JBI] Checklist for Case Series, and the Quality Assessment of Diagnostic Accuracy Studies-2 [QUADAS-2] [a revised tool for the quality assessment of diagnostic accuracy studies]).
- Summarize the findings within the current literature of IRDs genetics.
- Assess whether LRS has addressed key limitations of NGS, particularly in achieving complete coverage of IRD loci and detecting complex genetic variants that are challenging to identify with SRS.

Methods

Ethics Statement

This study is a systematic review that analyzes data obtained from published, peer-reviewed literature that had received prior ethical approval and participant consent by the original investigators. No human or animal samples were collected. Therefore, the institutional review board of Beirut Arab University determined that approval and informed consent were not required. This review adhered to the principles of the Declaration of Helsinki and followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Checklist (Supplementary file 1, available at www.ophthalmologyscience.org). The protocol was registered in the International Prospective Register of Systematic Review (CRD42024602173).

Information Sources and Search Terms

Three online databases were searched to identify articles: PubMed, Web of Science, and the Cochrane Database of Systematic Reviews. Searches were conducted on September 1, 2025, with no restriction on publication year. Articles published from 2001 onward were considered. Search terms were initially generated for PubMed and subsequently adapted for the other databases. These databases were systematically screened using the combination of LRS Medical Subject Headings terms. In PubMed, we used the following Boolean search string: ("long-read sequencing"[MeSH Terms] OR "third generation sequencing" OR "PacBio" OR "Oxford Nanopore" OR "single molecule real time sequencing" OR "SMRT sequencing" OR "nanopore sequencing") AND ("inherited retinal disease" OR "retinitis pigmentosa" OR "Leber congenital amaurosis" OR "cone-rod dystrophy" OR "Usher syndrome"). The complete search strategy with Boolean operators, filters, and results count is provided in Table S1, available at www.ophthalmologyscience.org.

All retrieved records were imported into EndNote (version 20). Automatic duplicate removal was applied, followed by manual verification in Excel. Searches were performed independently in duplicate by 2 researchers.

The population–intervention–comparator–outcome framework was used to develop the search terms: "population" being IRDs of all types, "intervention" being LRS, no "comparator" was used, and "outcome" being implications of genetic diagnosis/solving rate for IRDs. In the absence of a dedicated registry for rare eye conditions, search terms were curated using resources such as the Genetic and Rare Diseases Information Center, the National Eye Institute, Retina International, and the Irish Target 5000 Gateway to Vision. A manual search was also conducted using free text terms like "LRS" and "IRD" to identify additional relevant articles not captured in the initial search.

Inclusion and Exclusion Criteria

All studies focusing on the use of LRS in IRD genetics were included if they met the following criteria: (1) reports of all sorts (case reports, case series, clinical trials) that include IRD diagnosed genetically through LRS, even if they also address other genetic diseases; and (2) articles are written in English with full text available. Studies were excluded if (1) they did not report IRD variants; (2) the samples were not human (animal models); (3) they did not have the full text available; and (4) they did not utilize LRS technologies.

Screening was performed in duplicate by 2 independent reviewers (M.I. and A.C.; title/abstract, then full text). When discrepancies between authors arise, the principal investigator reviews the evidence and rationale provided by the initial authors and makes an independent decision. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses flow diagram (Fig 1) details the number of records at each stage, reasons for exclusion, and final inclusions.

Unit of Analysis

The primary unit of analysis was the genetic variant. Each distinct gene variant was considered as a single analytical entry, regardless of how many patients or studies reported it. When multiple gene variants were described in a single patient, each variant was extracted and analyzed separately at the variant level. Patient-level characteristics (e.g., phenotype, inheritance pattern) were linked to the corresponding variants but were not used as independent analytical units. To minimize double counting: (1) if the same variant was reported across multiple studies, it was counted only once in the descriptive synthesis, with all relevant references cited; (2) duplicated variants resulting from repeated reporting of the same individuals across studies were identified by searching for identical patient IDs, with shared author groups, recruitment centers, overlapping study periods, and similar cohort descriptions used as supporting evidence. Variants were cross-checked at the genomic coordinate and allele level to confirm uniqueness. No duplicate variants were identified for exclusion using this approach; and (3) patient-level counts (when presented) were used only to illustrate context (e.g., the number of individuals carrying a variant) and not as the primary unit of analysis. Variants classified as benign, likely benign, or variants of uncertain significance (VUS) were excluded from this study. Additionally, variants detected by NGS and subsequently confirmed by LRS without providing any added value (e.g., phasing, VUS reclassification) were not included. Conversely, variants initially detected by NGS for which LRS contributed additional information (e.g., improved characterization, phasing, or reclassification of VUS) were included in this study.

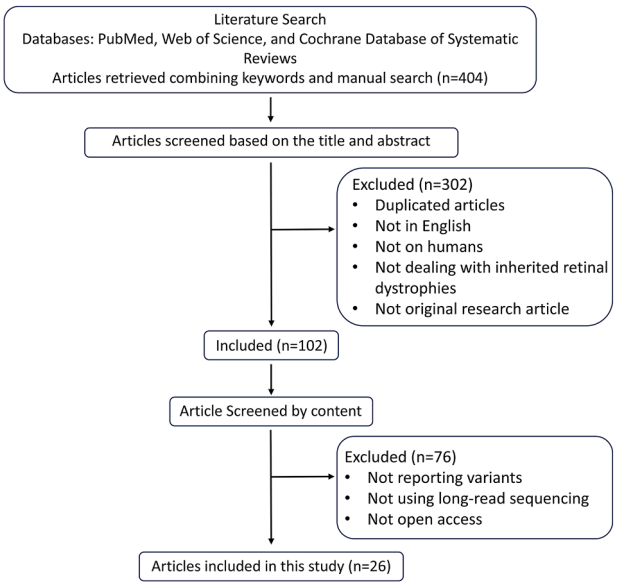

Figure 1. Flowchart illustrating the process for identifying eligible articles. The initial combined search retrieved 404 articles, which were then filtered down to leave 102 articles. After content screening, 76 additional articles were excluded, resulting in a final set of 26 articles published till September 1, 2025.

Data Extraction

Reference lists were screened for reverse citations, and a cited reference search was conducted on the Web of Science to identify potential forward citations. Data were extracted independently in duplicate using a standardized form. Extracted variables included (1) patient characteristics (number of patients, clinical diagnosis); (2) genetic findings (variant type, gene, affected region); (3) sequencing details (LRS platform, prior NGS use); and (4) publication characteristics (year, aim).

Critical Appraisal and Risk of Bias

Because the included studies encompassed different observational and diagnostic designs, we used design-specific quality assessment tools; specifically, the Murad's tool,[23] the JBI[24] Checklist for Case Series, and the QUADAS-2 (a revised tool for the quality assessment of diagnostic accuracy studies).[25]

Case reports and small case series ($n \leq 10$) were appraised with the Murad et al tool, which evaluates selection, exposure/outcome ascertainment, causality, and reporting domains. For larger observational or methodological cohorts, we applied the JBI Checklist for Case Series, which assesses inclusion clarity, recruitment methods, data completeness, and analytical transparency.

Comparative and diagnostic accuracy studies were evaluated using the QUADAS-2 framework, which examines bias and applicability across patient selection, index test validity, reference standard, and flow/timing domains. Because JBI and QUADAS-2 differ conceptually, their outputs were harmonized into a unified framework to enable direct comparison across all non−case-report studies.

Each tool's original domains were mapped conceptually onto 5 common categories: Selection, Test/Method Validity, Reference Standard/Confirmation, Flow & Timing, and Reporting & Analysis, followed by a global Overall Risk of Bias (RoB) rating. This harmonization preserved each tool's intent while allowing consistent synthesis of mixed-design evidence, an accepted methodological approach for systematic reviews that integrate observational and diagnostic literature. Each study was assigned a global rating of low, moderate, or high RoB using predefined rules: (1) low: all or most domains rated low, with no critical flaw; (2) moderate: minor methodological shortcomings or unclear items; and (3) high: $\geq$1 critical flaws or major reporting limitations.

Data Synthesis and Analysis

This study was conducted as a systematic review with descriptive synthesis; therefore, the results were narratively summarized to capture the scope and contribution of LRS to IRD genetics. The

Table 1. A Brief Description of the Studies Included (n = 26)

Authors	LRS Platform	Aim of Using LRS	NGS (Initial Sequencing)	Year	Reference
Reiner et al.	PacBio (targeted)	Identification of the precise deletion breakpoints	CMA testing and BBS gene panel	2018	26
Vaché et al.	ONT (targeted)	Characterization of a missed complex chromosomal rearrangement	MPS-based gene panel	2020	27
Fadaie et al.	PacBio (WGS)	Identification of hidden SVs	Targeted	2021	21
Ascari et al.	ONT (targeted-WGS)	Delineation of breakpoint junctions at the nucleotide level	WES, WGS	2021	18
Miller et al.	ONT (targeted)	Identification of missing disease-causing variant	WGS and targeted sequencing	2021	28
Sano et al.	ONT (WGS)	Identification of SVs in previously unsolved cases	Targeted	2022	29
Wu et al.	ONT (WGS)	Identification of the underlying genetic cause of the disease	WGS	2022	30
Sorrentino et al.	PacBio (targeted)	Identification of the disease-causing second hit in a genetically unsolved case	Targeted	2022	31
Jurkute et al.	ONT (targeted)	Characterization of splice variants	WGS, WES	2022	32
Nakamichi et al.	ONT (targeted)	Identification and phasing of variants	Exome panel	2023	20
Gupta et al.	ONT (targeted)	Identification and haplotyping variants from proband alone in the absence of familial data	Targeted	2023	33
McClinton et al.	ONT (targeted)	Characterization of a previously identified variant at nucleotide-level resolution	MIP panel targeting 100 IRD genes, WES	2023	34
Bonetti et al.	PacBio (targeted)	Detection of genetic variants in low-complexity region	Targeted	2023	35
AlAbdi et al.	PacBio (WGS)	Identification of the underlying genetic cause of the disease missed by exome sequencing	WES	2023	36
Yahya et al.	ONT-PacBio (targeted)	Sequencing RPGR-ORF15 locus of patients with IRDs	WES	2023	37
Fernández-Suárez et al.	ONT (targeted)	Identification of the disease-causing second hit in a genetically unsolved case/characterization of a previously detected CNV	Targeted	2024	38
Backlund et al	ONT (targeted)	Characterization of a mobile element insertion	Targeted and WES	2024	39
Varela et al.	ONT (targeted)	Characterization of the splicing effect of a variant	Targeted, WES, and WGS	2024	40
Maggi et al.	ONT (targeted)	Characterize and quantify aberrant splicing caused by variants in IRD genes	WES, LR-PCR, WGS	2024	41
Rodilla et al.	ONT (targeted)	Genetic screening of RPE65 locus	CES-WGS	2024	42
Chandrasekhar et al.	ONT (targeted)	Characterization of candidate splice-site variants	WGS	2024	43
Li et al.	PacBio (targeted)	Detection of variants causing bestrophinopathy in 2 families	WES, RPGR ORF15 site testing (in 1 patient)	2024	44
Nakamichi et al.	ONT (targeted)	Phasing, reclassification of VUS, detection of precise breakpoints of SV, detection of missing variants	Targeted	2024	45
Rodilla et al.	ONT (WGS)	Evaluation of the clinical applications of LR-WGS (variants confirmation and SVs definition)	WGS	2025	46
Fabian-Morales et al.	PacBio (WGS)	Identification of the underlying genetic cause of the disease missed by NGS	Gene panel-ES	2025	47
Capasso et al.	PacBio (targeted lrcDNA sequencing)	Identification of the underlying genetic cause of IRDs in unsolved IRD cases	Short-read sequencing	2025	48

BBS = Bardet–Biedl syndrome; CES = clinical exome sequencing; CMA = chromosomal microarray; CNV = copy number variant; ES = exome sequencing; lrcDNA = long-read complementary DNA; IRD = inherited retinal dystrophy; LR-PCR = long-range polymerase chain reaction; LRS = long-read sequencing; MIP = molecular inversion probes; MPS = massive parallel sequencing; NGS = next-generation sequencing; ONT = Oxford Nanopore Technologies; PacBio = Pacific Biosciences; SV = structural variant; VUS = variants of uncertain significance; WES = whole-exome sequencing; WGS = whole-genome sequencing.

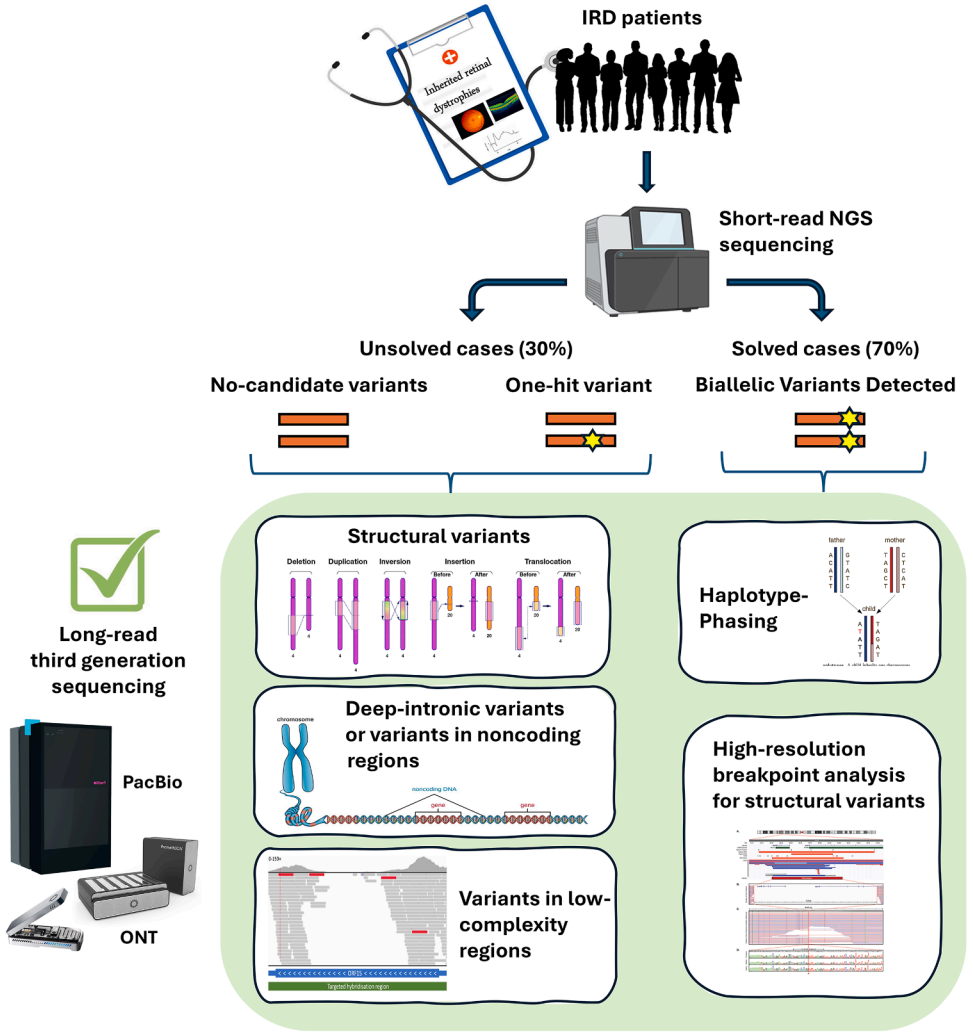

Figure 2. Identifying causal variants in IRD cases: challenges of SRS and the potential of LRS. IRD = inherited retinal dystrophy; LRS = long-read sequencing; NGS = next-generation sequencing; ONT = Oxford Nanopore Technologies; PacBio = Pacific Biosciences; SRS = short-read sequencing.

primary focus of this review was to qualitatively assess the added value of LRS over NGS in detecting or characterizing disease-causing variants. Specifically, we synthesized evidence on how LRS contributed to detecting causal variants missed by NGS, refining the characterization of previously identified variants through precise breakpoint mapping, and providing phasing information or enabling reclassification of variants of VUS. The secondary outcomes included describing the spectrum of genes and variant types identified using LRS and the methodological approaches adopted across studies. The synthesis emphasizes qualitative trends and methodological insights rather than quantitative effect sizes.

Results

Characterization of Included Studies

The literature search identified records from PubMed (n = 251), Web of Science (n = 113), the Cochrane Database of Systematic Reviews (n = 14), and manual searching (n = 26) (Fig 1). After content screening, a total of 26 articles were included. These articles were published between 2018 and September 1, 2025 (Table 1).

Based on existing literature, LRS reportedly may (1) detect causal variants missed by NGS; (2) provide more detailed analyses of previously identified variants with precise breakpoints identification; and (3) construct haplotype phasing (Fig 2). The latter advantage is especially valuable when parental samples are unavailable.[49]

The classification of study designs and the corresponding RoB assessment tools are presented in Table S2, available at www.ophthalmologyscience.org. Among the included articles, 16 were case reports or case series, and their quality was evaluated using Murad's tool, which assesses selection, exposure and outcome, causality, and reporting quality (Table S3, available at www.ophthalmologyscience.org). Included studies demonstrated a careful and representative selection of participants, focusing on genetic and clinical contexts. Participants were diverse, reflecting various genetic and phenotypic variability, such as BBS, RP, and

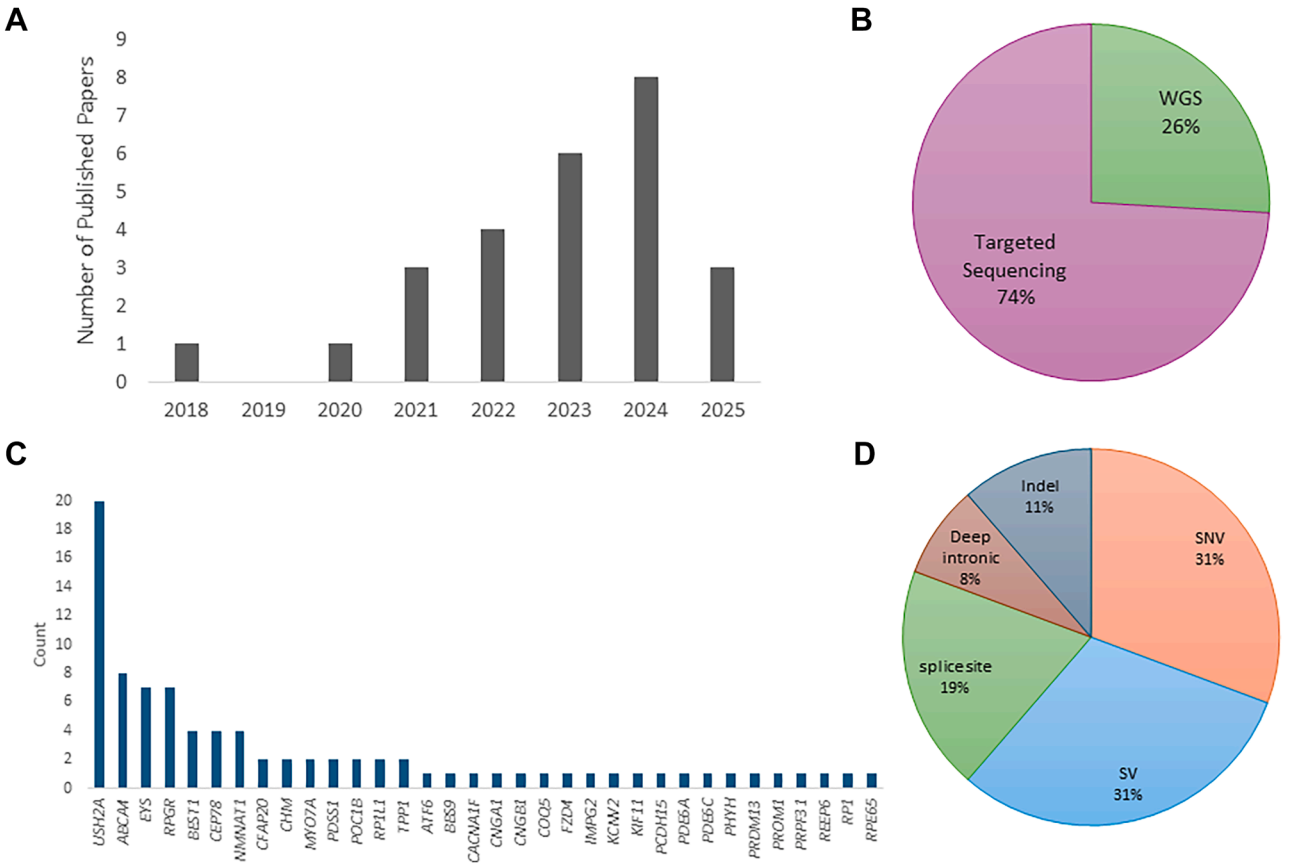

Figure 3. Overview of variants identification techniques across different articles. (**A**) Number of articles using LRS for IRD genetics research up to September 1, 2025; (**B**) LRS methods applied for variant identification (WGS and targeted sequencing); (**C**) IRD-associated genes in which variants were detected using LRS; (**D**) types of variants (pathogenic/likely pathogenic) identified through LRS. IRD = inherited retinal dystrophy; LRS = long-read sequencing; SNV = single nucleotide variant; SV = structural variant; WGS = whole-genome sequencing.

other IRDs with different inheritance patterns. Rigorous inclusion and exclusion criteria were implemented to ensure the reliability of conclusions. Many studies included patients with unsolved genetic diagnoses or challenging cases that required advanced sequencing methods, ensuring their relevance to clinical challenges. The articles employed PacBio or ONT technologies to correlate the genotype—phenotype associations. The reliability of these methods was emphasized in identifying complex genetic variants, such as SVs, insertions, deletions, and splice-altering variants, which are often missed by traditional SRS approaches. The studies also underscored the ability of LRS to achieve complete coverage of complex genomic loci, such as *RPGR* and *USH2A*. These methods were validated through accuracy rates and their effectiveness in resolving cases that had remained unsolved with conventional sequencing technologies. Adequate follow-up periods enabled the validation of findings through cosegregation analyses, functional assays, or transcriptomic investigations. Analyzed articles highlighted the utility of LRS in identifying causative variants in previously unsolved cases, particularly for conditions with high genetic heterogeneity. The ability to phase variants and reconstruct haplotypes provided additional insights into inheritance patterns. In terms of clarity, the studies were generally characterized by clear and transparent reporting. Methods and findings were described sufficiently to ensure reproducibility, including thorough documentation of sequencing protocols, variant annotations, and bioinformatics pipelines. Limitations such as sequencing errors, gaps in genomic coverage, and challenges with data analysis were explicitly acknowledged and discussed. In conclusion, the quality assessment using Murad's tool underscores the transformative potential of LRS in advancing genetic diagnostics for IRDs, highlighting its strengths in resolving complex genetic cases while acknowledging its limitations, such as sequencing errors and computational demands.

The remaining 10 studies were non—case-report designs, comprising 4 observational/methodological cohort studies and 6 diagnostic/comparative studies. These were appraised using design-specific tools (JBI or QUADAS-2). All were rated as having a low overall RoB, primarily due to the absence of quantitative diagnostic accuracy metrics such as sensitivity or confidence intervals.

The JBI-assessed studies showed strong methodological validity, complete data reporting, and orthogonal variant

Table 2. Pathogenic and Likely Pathogenic Variants Detected or Characterized by LRS*

Gene	Variant (as in Paper)	Type	Status	Size of Implicated Regions	Clinical Diagnosis	Missed by NGS	Improvement by LRS	Family/Patient ID	Number of Patients	Reference
EYS	NM_001142800.1:c.3443+14 421_5927+13 006delinsTCAT	SV	Htz	Deletion: ~376 kb (exon 23–28) and insertion: 4bp	RP	Yes	Identification of missing SV	OPH641	1	29
EYS	NM_001142800.1:c.6079–30740_7055+41631delinsCATAAT	SV	Htz	Deletion: ~395 kb (exon 30–35) and insertion: 6bp	RP	Yes	Identification of missing SV	OPH861	1	29
EYS	Chr6 (GRCh37):g.64430524_64430525ins352, NM_001142800.2:c.9402_9403ins[[JQ403527.1:g.57_367];9388_9402])	SV	Htz	~ 352 bp insertion (AluYa5 insertion in exon 43)	RP	Yes	Identification of missing SV "Second hit"	Family A	1	38
EYS	Chr6 (GRCh37):g.64764235_64820592 del, NM_001142800.2:c.6425-28697_6725 + 11996del)	SV	Htz	~56.4 kb (exon 32-33)	RP	No	Characterization of CNV	Family A	1	38
EYS	NM_001292009.1: g.65529289_65636754del	SV	Hom	107 kb (exon 16-21)	RP	No	Characterization of the breakpoints at nucleotide resolution	Case 3	1	34
EYS	c.3569-1312_3684+11593del	SV	Hom	13 kb (deletion of exon 24)	RP	Yes	Identification of missing variants	Case 2	1	47
BBS9	Deletion (chr7:33130616–33203409)	SV	Hom	72.8 kb (exons 1-4 and the entire RP9 gene)	BBS	No	Precise breakpoints delineation	-	1	26
CEP78	The left breakpoint is located at (hg38) chr9:78228782, whereas the right breakpoint at chr9:78244762. The inverted segment spans chr9:78234546–78234844 [nearby an L1ME3Cz repetitive element (chr9:78234521–7823634902)].	SV	Hom, Htz	15 kb (exons 1–5)	CRDHL	No	Precise breakpoints delineation and characterization	F1, F3	2	18
CEP78	(13 reference sequence reads, 21 deletion reads) deletion spanning the region chr9:78096930–78331887	SV	Htz	235 kb (total gene deletion of CEP78 and PSAT1)	CRDHL	No	Precise breakpoints delineation and characterization	F2	1	18
CEP78	c.1206-2A>C	Splice site	Htz		RCD	No	Phasing variants	Subject 20	1	45
CEP78	exons 1–5 were deleted (chr9:78,228,783–78,244,408)	SV	Htz	exons 1–5 were deleted + upstream of the gene	RCD	No	Phasing variants and epigenetic profiling (base methylation information)	Subject 20	1	45
PRDM13	chr6: g.99932464-100067110dup, NC_000006.11	SV	Htz	134.6 kb (entire CCNC and PRDM13 genes and a DNase 1 hypersensitivity site in the MCDR1 locus)	NCMD	No	Identification of variant	-	2	30
RP1L1	NM_178857.6:c.4026_4027insAC AGA AGA AGG GCTGCA AGA AGA GGG GGTGC AGT TAG AGG AAACTA AAACAG AAG AAG GGC TGC AAG AAG AGG GGG TGC AGT TA GAG GAA ACTAAA ACAGAAGAAGGG CT GCA AGA AGAGGG GGTGCAGTTAGA GG GGACTA AA;p.Glu1343delins ThrGluGlulyLeuGlnGluGlu GlyValGlnLeuGluGluThr LysThrGluGluGlyLeuGln GluGluGlyValGlnLeuGlu GluThrLysThrGluGluGly LeuGlnGluGluGlyValGln LeuGluGlyThrLys Glu	SV	Htz		LCA	Yes	Identification of missing variant	F3981	2	36

(*Continued*)

Table 2. (Continued.)

Gene	Variant (as in Paper)	Type	Status	Size of Implicated Regions	Clinical Diagnosis	Missed by NGS	Improvement by LRS	Family/Patient ID	Number of Patients	Reference
RP1L1	NM_178857.6:c.3970_3971insGGA CTA AAG TAA TAG AAG GGC TGC AAG AAG AGA GGG TGC AGT TAG AGG:p.Glu1324delinsGly ThrLysValIleGluGlyLeuGln GluGluArgValGlnLeuGluGlu	SV	Htz		LCA	Yes	Identification of missing variant	F3981	1	36
PRPF31	NM_015629.4: g.54617206_54619550delinsG	SV	Htz	2.4 kb (exon 1 of PRPF31 as well as exons 2 and 3 of the neighboring gene TFPT)	RP	No	Characterization of the breakpoints at nucleotide resolution	Case 4	1	34
PCDH15	hg38:chr10:g.[(54170766_ 54170947)(54171162_54171211) del;(54171162_54171211) (58862555_58862736) inv;(58862291_58862340)_ (58862555_58862736)dup]	SV	Htz	4.6 Mb paracentric inversion (PCDH15-LINC00844 and BICC1-PCDH15 fusion transcripts)	Usher syndrome type 1	Yes	Complex chromosomal rearrangement characterization	Family S331/II-1	1	27
RP1	c.2106_2107insL1	SV	Htz	5.6 kb LINE-1 transposon insertion in exon 4	RP	No	Characterization of a LINE-1 insertion	-	2	39
CNGA1	NM_001142564.1: g.47931965_47946798del	SV	Hom	14 kb (exon 6-10)	RP	No	Characterization of the breakpoints at nucleotide resolution	Case 1	1	34
CNGB1	NM_001286130.2: g.57937451_57946811del	SV	Hom	9.4 kb (exon 25–27)	RP	No	Characterization of the breakpoints at nucleotide resolution	Case 2	1	34
CACNA1F	c.2239+5C>G	Splice site	Htz		RD	No	Functional characterization	Patient ID 4	1	41
PDE6C	c.864+1G>A	Splice site	Hom		COD	No	Functional characterization	Patient ID 9	1	41
ATF6	c.1534-9A>G	Splice site	Htz		ACHR	No	Functional characterization	Patient ID 3	1	41
RPGR	c.1415-9A>G	Splice site	Htz		RP	No	Functional characterization	Patient ID 14	1	41
POC1B	c.677-2A>G	Splice site	Htz		CRD	No	Functional characterization	Patient ID 10	1	41
POC1B	c.1033-327T>A	Deep intronic	Htz		CRD	No	Functional characterization	Patient ID 10	1	41
KIF11	c.1875+2T>A	Splice site	Htz		EVR	No	Functional characterization	Patient ID 8	1	41
PROM1	c.2490-2A>G	Splice site	Htz		STGD	No	Functional characterization	Patient ID 12	1	41
CHM	c.1413G>C	SNV	Htz		RP/CHM	No	Functional characterization	Patient ID 5	1	41
FZD4	c.313A>G	SNV	Htz		EVR	No	Functional characterization	Patient ID 6	1	41
IMPG2	c.3423-7_3423-4del	Splice site	Htz		MD	No	Functional characterization	Patient ID 7	1	41
REEP6	c.517G>A	SNV	Htz		RP	No	Functional characterization	Patient ID 13	1	41
PDSS1	c.468-25 A > G	Splice site	Htz		RP	No	Characterization of splice-site variants	Family 5	1	32

Table 2. (Continued.)

Gene	Variant (as in Paper)	Type	Status	Size of Implicated Regions	Clinical Diagnosis	Missed by NGS	Improvement by LRS	Family/Patient ID	Number of Patients	Reference
PDSS1	c.722-2 A > G	Splice site	Htz		RP	No	Characterization of splice-site variants	Family 3	1	32
COQ5	c.682-7 T > G	Splice site	Htz		RP	No	Characterization of splice-site variants	Family 11, Family 12	2	32
PHYH	c.678+5G>T	Splice site	Hom, Htz		Isolated RP or syndromic IRD (Refsum disease)	No	Characterization of splice-site variant	GC20955, GC15871, GC28415, GC20417	4	40
CHM	c.1510+693_1510+ 694ins1414-1244_1510+ 402inv	SV	Hem	1752 bp inverted duplication including exon 12 and surrounding regions	Choroideremia	Yes	Identification and characterization of the hidden inverted duplication	Family A	1	21
BEST1	c.202T>C	SNV	Htz		Autosomal recessive bestrophinopathy	-	Identification of variant	Family A	1	44
BEST1	c.488T>G	SNV	Htz		Autosomal recessive bestrophinopathy		Identification of variant	Family A	1	44
BEST1	c.867+97G>A	Deep intronic	Htz		Autosomal recessive bestrophinopathy	Yes	Identification of variant	Family B	1	44
BEST1	c.584C>T	SNV	Htz		Autosomal recessive bestrophinopathy	No	Identification of variant	Family B	1	44
RPE65	c.1067dup, p.(Asn356Lysfs*9)	Indel	Htz		LCA/EOSRD	Yes	Identification of missing variants	P5	1	31
RPGR	c.2074G>T p.(Gly692*), ChrX:38146178C>A (hg19)	SNV	Htz		RP	Yes	Identification of missing variant	5550	1	37
RPGR	c.2041_2042delAA, p.(Lys681Glyfs*2), ChrX:38146210_38146211del (hg19)	Indel	Hom		RP	Yes	Identification of missing variant	3219	1	37
RPGR	c.2323_2324delAG, p.(Arg775Glufs*59), ChrX:38145933_38145934del (hg19)	Indel	Hom		RP	Yes	Identification of missing variant	3685	1	37
RPGR	NM_001034853.2:c.2919_2940dup	Indel	Htz		RP or CD	Yes	Identification of variants in low-complexity regions	-	1	35
RPGR	NM_001034853.2:c.2820_2841dup	Indel	Htz		RP or CD	Yes	Identification of variants in low-complexity regions	-	2	35
RPGR	NM_001034853.2:c.3262_3263insA	Indel	Htz		RP or CD	Yes	Identification of variants in low-complexity regions	-	1	35
TPP1	c.837C>G, p.Tyr279Ter	SNV	Htz		Syndromic IRD	No	Allelic architecture of variants	Family 2	2	45
TPP1	c.508+4T>C	Splice site	Htz		Syndromic IRD	No	Allelic architecture of variants	Family 2	2	45
ABCA4	~1500 bp transposableelement insertionin intron 1	SV	Htz	~1500 bp composite retrotransposable element insertion consisting of AluJ (SINE) and partial L2a, L2c, L2d2, and L1HS (LINEs) mapping within intron 1	Stargardt	Yes	Identification of missing variant	S025	1	28
ABCA4	c.3113C>T, p.Ala1038Val	SNV	Htz	-	Stargardt	-	Allelic architecture	Subject 13	1	45
ABCA4	c.1622T>C, p.Leu541Pro	SNV	Htz	-	Stargardt	-	Allelic architecture	Subject 13	1	45
ABCA4	c.2041C>T, p.Arg681Ter	SNV	Htz	-	Stargardt	-	Allelic architecture	Subject 13	1	45
ABCA4	c.5603A>T, p.Asn1868Ile	SNV	Htz	-	Stargardt	-	Allelic architecture	Subject 14	1	45

(*Continued*)

Table 2. (Continued.)

Gene	Variant (as in Paper)	Type	Status	Size of Implicated Regions	Clinical Diagnosis	Missed by NGS	Improvement by LRS	Family/Patient ID	Number of Patients	Reference
ABCA4	c.2588G>C, p.Gly863Ala	SNV	Htz	-	Stargardt	-	Allelic architecture	Subject 14	1	45
ABCA4	c.5461-10T>C	Splice site	Htz	-	Stargardt	-	Phasing of variants	Subject 14, subject 15	2	45
ABCA4	c.3413T>C, p.Leu1138Pro	SNV	Htz	-	Stargardt	-	Phasing of variants; reclassification of VUS	Subject 15	1	45
PDE6A	c.1646T>C, p.Leu549Pro	SNV	Htz	-	RP	-	Phasing of variants; reclassification of VUS	Subject 16	1	45
USH2A	c.6159del	Indel	Htz		USH2	No	Phasing variant	Family 3	2	45
USH2A	c.4106C>T	SNV	Htz		USH2	No	Phasing variant	Family 3	2	45
USH2A	c.9270C>A	SNV	Htz	-	USH2	No	Phasing variant	Family 3	2	45
USH2A	c.12067-2A>G	Splice site	Htz	-	USH2	No	Identification and phasing of variants	Subject 17	1	45
USH2A	c.3299dup, p.Glu1100GlufsTer8	Indel	Htz	-	USH2	No	Identification and phasing of variants	Subject 17	1	45
USH2A	c.14803C>T, p.Arg4935Ter	SNV	Htz	-	USH2	No	Phasing of variants	Subject 18	1	45
USH2A	c.141314-3169A>G	Deep intronic	Htz	-	USH2	Yes	Identification of a missing variant; phasing of variants	Subject 18	1	45
USH2A	c.14369A>C, p.Gln4790Pro	SNV	Htz	-	USH2	No	Phasing of variants	Subject 19	1	45
USH2A	a large deletion encompassing exons 42 and 43 (Chr1:215,875,713 −215,884,830)	SV	Htz	A large deletion encompassing exons 42 and 43	USH2	Yes	Identification of a missing variant; phasing of variants	Subject 19	1	45
USH2A	c.9959-2971C>T	Deep intronic	Htz	Pseudoexon insertion of 113 bp following exon 50	USH2	No	characterization of splice defects	Patient 1	1	43
USH2A	c.8682-654C>G	Deep intronic	Htz	Pseudoexon inclusion of 130 bp.	RP	No	Characterization of splice defects	Patient 4	1	43
USH2A	c.9055+389G>A	Deep intronic	Htz	78 bp pseudoexon inclusion	USH2	No	Characterization of splice defects	Patient 5	1	43
USH2A	c.1256G > T, p.Cys419Phe	SNV	Htz		Usher syndrome	No	Haplotype reconstruction and phased variant identification	Subject 1 Subject 2	2	20
USH2A	c.1111_1112del, p.Ile371Phefs*3	Indel	Htz		Usher syndrome	No	Haplotype reconstruction and phased variant identification	Subject 1 Subject 2	2	20
USH2A	c.10820A>C (p.His3607Pro)	SNV	Htz		Usher syndrome	No	Haplotyping from proband alone in the absence of familial data	Case 5	1	33
USH2A	c.14995A>G (p.Thr4999Ala)	SNV	Htz		Usher syndrome	No	Haplotyping from proband alone in the absence of familial data	Case 5	1	33
USH2A	c.2139C>T; p.(Gly713 =)	SNV	Htz	Partial exon 12 skipping	USH2	No	Characterization of splice defects	Patient 2	1	43
USH2A	c.3812-3_3837dup p.(Met1280Ter)	Indel	Htz	Exon 18 skipping and exon 18/partial exon 19 skipping	RP	No	Characterizations of splice defects	Patient 3	1	43
USH2A	c.4885+740A>T	Deep intronic	Hom, Htz		Usher	Yes	Identification of a missing variant	Case 1 Case 3	2	47

Table 2. (Continued.)

Gene	Variant (as in Paper)	Type	Status	Size of Implicated Regions	Clinical Diagnosis	Missed by NGS	Improvement by LRS	Family/Patient ID	Number of Patients	Reference
USH2A	c.4628-2298_6325+22604dup (DUP:chr1:216023827-216099511, hg38 ref-erence genome)	SV	Htz	75.6 kb duplication of exons 22–32	Usher	No	Phasing of variants	Case 3	1	47
EYS	NM_001142800.2:c.2137+1990_3443+74729del (NC_000006.12:g.64738649_65055624del)	SV	Htz	316.9 kb	RP	No	Precise breakpoints definition of SV	C001VXB	1	46
KCNV2	NM_133497.4:c.1357-5153_*898del (NC_000009.12:g.2724293_2730625del)	SV	Htz	6.3 kb	Retinal dystrophy	No	SV refinement, defining exact deletion size/breakpoints	C001VXC	1	46
CFAP20	NM_013242.3:c.257A >G; p.Tyr86Cys	SNV	Htz		Syndromic RP	Yes	Identification and phasing	C002312	1	46
CFAP20	NM_013242.3:c.277-207_*57060del (NC_000016.10:g.58056967_58115666del)	SV	Htz	58.7 kb (CFAP20 deletion)	Syndromic RP	Yes	Identification and phasing	C002312	1	46
MYO7A	NM_000260.3:c.1657C > T	SNV	Htz		Usher syndrome type I	Yes	Identification and phasing of variants	C00231G	1	46
MYO7A	NM_000260.3:c.5945-60_*10804del; chr11:77208637-77225500del	SV	Htz	16.9 kb deletion	Usher syndrome type I	Yes	Identification and phasing of variants	C00231G	1	46
NMNAT1	NM_022787.4: c.769G>A	SNV	Htz		LCA	No	Validation of expressed allele; phasing of alleles	P-6, P-7, P-8, P-9	4	48
NMNAT1	c.-57+1del	Splice site	Htz		LCA	No	Characterization and reclassification of VUS	P-6	1	48
NMNAT1	c.-57G>A	SNV	Htz		LCA	No	Characterization and reclassification of VUS	P-7	1	48
NMNAT1	NC_000001.11:g.9972228_9972229ins[G; NC_000006.12:g.122847777_122850290inv; A38; GACTAGAGAACC]	SV	Htz	Insertion of the complete SVA_F retrotransposon sequence (2691 bp) in intron 2	LCA	Yes	Identification and full characterization of a mobile element insertion	P-8, P-9, P-10	3	48

ACHR = achromatopsia; BBS = Bardet–Biedl syndrome; CD = cone dystrophy; CHM = choroideremia; CNV = copy number variant; COD = cone dystrophy; CRD = cone-rod dystrophy; CRDHL = cone-rod dystrophy with hearing loss; EOSRD = early-onset severe retinal dystrophy; EVR = exudative vitreoretinopathy; Hem = hemizygous; Hom = homozygous: Htz = heterozygous; LCA = Leber congenital amaurosis; LRS = long-read sequencing; MD = macular dystrophy; NGS = next-generation sequencing; NMCD = North Carolina macular dystrophy; RCD = rod-cone dystrophy; RD = retinal dystrophy; RP = retinitis pigmentosa; SNV = single nucleotide variant; STGD = stargardt disease; SV = structural variant; SVA_F = SINE–VNTR–Alu; USH2 = type 2 Usher syndrome; VUS = variants of unknown significance.

*Benign, likely benign or VUS were not included in this table. Variants detected by NGS and subsequently confirmed by LRS, without yielding any additional benefit (phasing, VUS reassignment, etc), were not considered. Part of the variants, particularly SNVs, were detected by short-read sequencing; however, they were included in this study when LRS provided additional information for their characterization, phasing, or reclassification of variants of uncertain significance.

confirmation, but all were based on targeted or research-enriched cohorts (Table S4, available at www.ophthalmologyscience.org). The QUADAS-2—assessed studies demonstrated high analytical rigor and complete flow between index and reference tests but were limited by selective recruitment and qualitative yield reporting rather than formal diagnostic accuracy estimates (Table S4). No study was classified as high RoB, and none exhibited incomplete reporting or methodological errors that would preclude inclusion in the synthesis. Across all non—case-report studies, the technical and analytical robustness of LRS workflows was consistently high, with selection bias remaining the predominant methodological concern (Table S4).

Overview of LRS Application in IRDs

Our results revealed an apparent increase in publications using LRS for IRD genetics over the last years (Fig 3A). Most LRS applications were targeted sequencing (74%, Fig 3B). This predominance is expected because targeted LRS is more frequently used than WGS due to several practical and technical advantages. Targeted LRS is more cost-effective and scalable, and its ability to phase variants can enhance its diagnostic utility.[50] Table 2 illustrates the contribution of LRS to IRD genetics, including its ability to detect and precisely delineate genetic variants. In total, 88 variants were analyzed in our study. Of these, single nucleotide variants and SV each accounted for 31%, followed by splice-site variants (19%), insertions and/or deletions (indels) at 11%, and deep intronic at 8% (Fig 3D). Part of the variants was detected by SRS; however, they were included in this study when LRS provided additional information for their characterization, phasing, or reclassification of VUS. Only variants classified as pathogenic or likely pathogenic were considered.

Our analysis revealed that LRS may expand the genetic landscape of IRDs by identifying and refining variants in several IRD-associated genes where NGS has failed (Fig 3C). Particularly, LRS contributed to the identification or refined characterization of pathogenic variants in 34 genes, including *USH2A* (23%), *ABCA4* (9%), *EYS,* and *RPGR* (8% each) (Fig 3C). Notably, the reviewed studies aimed to validate the ability of LRS to detect variants or to improve its capability in variant detection. An overall synthesis of study features and major conclusions is outlined in Table 3.

Discussion

Long-read sequencing overcomes key limitations of SRS in the genetic diagnosis of IRDs. Although all studies were methodologically rigorous, several diagnostic studies reported diagnostic yield qualitatively, for example, stating that LRS identified additional pathogenic or SVs without presenting quantitative diagnostic accuracy metrics, such as sensitivity, specificity, false discovery rate, or corresponding confidence intervals. The absence of such statistics limits the precision and reproducibility of comparisons across technologies (e.g., LRS vs NGS). This bias source might reduce external generalizability.

At least 48% of deletions and 83% of insertions are estimated to be missed by multiple short-read calling algorithms,[51,52] underscoring the value of LRS for SVs detection.[17] In 2 previously unsolved RP cases with a single detected *EYS* variant, LRS identified 2 large exon-overlapping deletions, providing the previously missing second allele and thereby completing the genetic diagnosis.[29] The breakpoints of both SVs were located within repetitive sequences.[29] Similarly, Fabian-Morales et al[47] demonstrated the utility of LRS in resolving previously inconclusive NGS results in 3 patients, identifying and characterizing SVs in 2 cases: a homozygous intragenic deletion of *EYS* exon 24 and a multiexon duplication spanning *USH2A* exons 22—32 with a deep intronic variant. In a case of BBS, clinical chromosomal microarray and targeted *BBS* gene panel sequencing identified a homozygous deletion of *BBS9* exons 1-4 within a 17.5-Mb region of homozygosity on 7p14.2-p21.1.[26] Single-molecule real-time sequencing was able to identify the precise nucleotide breakpoints and map a 72.8 kb deletion consistent with nonhomologous end joining.[26] In a patient with Usher syndrome type 1, massive parallel sequencing—based gene panel testing identified a multiexon *PCDH15* deletion but failed to detect a second variant.[27] Long-read sequencing deciphered a 4.6 Mb paracentric inversion on chromosome 10 generating 2 fusion transcripts (*PCDH15-LINC00844* and *BICC1-PCDH15)* involving highly homologous human endogenous retrovirus subfamily H transposable elements, and a segmental duplication associated with a deletion.[27] This complex SV, undetectable by standard methods, demonstrates the capability of LRS to resolve large inversions and repeat-mediated rearrangements in IRDs. In a large Dutch family with X-linked choroideremia described by van den Hurk et al[21] in 2003, an aberrant splicing of exon 12 in the *CHM* gene was detected, although the underlying genomic defect remained unidentified. Long-read sequencing combined with optical genome mapping enabled the detection and the complete characterization of a 1752 bp intragenic inverted duplication, uncovering the hidden pathogenic SV.[21] Additionally, LRS was able to detect and map a novel 134.6-kb tandem duplication on chromosome 6 encompassing the entire *CCNC* and *PRDM13* genes, as well as a DNase I hypersensitivity site in the *MCDR1* locus in a Chinese family with North Carolina macular dystrophy.[30] Long-read sequencing identified a compound heterozygous SV in *RP1L1* gene that was missed by WES, demonstrating the added value of LRS for precise variant mapping.[36]

Long-read sequencing accurately resolved several SVs that SRS could not fully characterize. It precisely defined a 107 kb *EYS* deletion (exons 16-21), a novel 14 kb homozygous *CNGA1* deletion (exons 6-10), a 9.4-kb *CNGB1* deletion (exons 25—27), and a 2.4 kb heterozygous deletion encompassing *PRPF31* exon 1 and *TFPT* exons 2 and 3.[34] Another study demonstrated that LRS enables precise delineation of SVs in IRDs.[46] Among relevant findings,

Table 3. Summary of Main Findings

Aspect	Summary of Findings (Aggregate across Studies)
Number of studies included	26 (published between 2018 and 2025) Searched on September 1, 2025
Study types and designs	Mainly case reports, small case series, and cohort studies
Total number of patients	81 individuals
Number of IRD genes investigated	34 genes (88 variants)
Most frequently analyzed genes	USH2A (23%), ABCA4 (9%), EYS (8%), RPGR (8%)
Main variant classes identified	SNV (31%), SV (31%), splice site (19%), indels (11%), deep intronic (8%)
Key outcomes reported	LRS can - Provide complete coverage of disease loci, including repetitive or GC-rich regions that are challenging for SRS - Detect hidden variants missed by NGS, including mobile elements and complex rearrangements - Provide precise breakpoints definition of SV, enabling detailed characterization of variants - Construct haplotype phasing, even in the absence of parental samples - Reclassify VUS
Overall interpretation	Early evidence supports LRS as a complementary approach that expands variant detection and refines molecular diagnosis in IRDs.

GC = guanine−cytosine; IRD = inherited retinal dystrophy; LRS = long-read sequencing; NGS = next-generation sequencing; SNV = single nucleotide variant; SRS = short-read sequencing; SV = structural variant; VUS = variant of unknown significance.

LRS refined the breakpoints of an *EYS* multiexon deletion and a *KCNV2* SV, providing their exact sizes.[46] Ascari et al[18] used LRS to characterize 2 SVs in *CEP78*: a ∼15-kb complex deletion−inversion−deletion affecting exons 1−5 (homozygous in one individual and heterozygous in another) and a heterozygous ∼235-kb deletion encompassing *CEP78* and *PSAT1*, occurring in trans with another *CEP78* variant.

Although several tools exist for identifying transposable elements from SRS data, these events are frequently overlooked unless specifically targeted.[39,53] Besides, multikilobase elements characterization is impossible using short reads.[39] Detection of mobile elements is hindered by the inherent limitations of short-read approaches in spanning long, repetitive regions, the limited integration of specialized computational methods into routine diagnostics able to recognize these events, and the small number of reported pathogenic insertional events to date.[48,54] These challenges highlight the added value of LRS, which can detect such events and capture complex SVs that might otherwise be overlooked. For example, in an individual with Stargardt disease, SRS identified only 1 pathogenic *ABCA4* variant, failing to detect a second hit. Long-read sequencing revealed a 1500 bp retrotransposon insertion within intron 1 of *ABCA4*.[28] Long-read sequencing identified a hidden AluYa5 insertion in exon 43 of *EYS*, the first reported in this gene, and precisely defined the breakpoints of a large deletion, findings missed by SRS.[38] In another case, targeted adaptive nanopore sequencing enabled precise definition of a novel 5.6 kb LINE-1 transposable element insertion in exon 4 of *RP1*, in a Finnish family with dominant RP.[39] Besides, LRS enabled the identification of a deleterious insertion of a SINE−VNTR−Alu (SVA_F) retrotransposon in intron 2 of *NMNAT1,* which was not detected by SRS due to its sequence complexity.[48]

The *RPGR* isoform containing ORF15 harbors >70% of variants causing X-linked RP.[55] Several variant detection methods have been used since the *RPGR*-ORF15 locus was identified as a variant hotspot, including direct Sanger sequencing, cloning the PCR product followed by Sanger sequencing, and direct sequencing with nested primers.[37,55] Amplifying the *RPGR*-ORF15 locus using standard PCR and Sanger sequencing is challenging due to polymerase enzymes slipping or stopping caused by hairpins and other structures within the repetitive region.[37] This complexity and common polymorphic indels complicate sequence alignment.[37]

Short-read sequencing is unsuitable for sequencing the *RPGR*-ORF15, as coverage drops sharply in exon 15, often resulting in inaccurate variant detection.[35] This may lead to false results and misannotation of genetic variants in databases such as the Database of Single Nucleotide Polymorphisms and the Clinical Variant Database, impeding accurate genetic diagnosis and the development of effective treatment plans.[35] In contrast, PacBio LRS has shown much higher coverage and accuracy for the *RPGR*-ORF15 exon, enabling the detection of variants missed by conventional NGS.[35] In 8 unrelated patients, PacBio sequencing revealed 8 *RPGR* variants undetected

by NGS, with 4 classified as likely pathogenic.[35] Additionally, Yahya et al[37] demonstrated that LRS successfully reads through the hard to sequence *RPGR*-ORF15 region, generating high-quality reads and sufficient cumulative read depth to detect pathogenic *RPGR* variants.

Variants in the *RPE65* gene are responsible for 0.6%−6% of RP cases and 3%-16% of Leber congenital amaurosis/early-onset retinal dystrophy cases.[56] *RPE65*-IRD cases present significant challenges, including the complexity of genetic diagnosis and the determination of gene therapy eligibility.[56] The possibility of a "double-hit" etiology or phenotype-modifying coexisting variants complicates the clinical picture, necessitating further research to understand their implications.[56] Long-read sequencing technologies offer comprehensive coverage crucial for identifying additional variants or "second hits," essential for a conclusive diagnosis.[31] In patients with Leber congenital amaurosis or RP, PacBio-based LRS pipeline (PacMAGI) enabled full *RPE65* gene coverage, confirming all NGS-detected variants.[31] In one case, PacMAGI revealed a second pathogenic allele, demonstrating the utility of LRS for detecting hidden variants missed by NGS due to low coverage.[31] Rodilla et al[42] applied Cas9-targeted LRS to 5 IRD patients carrying a single heterozygous *RPE65* variant unresolved by prior clinical exome sequencing. The method achieved high coverage of the entire *RPE65* region and confirmed all previously identified coding variants.[42] No additional causative *RPE65* variant in any of the 5 cases were found, ruling out biallelic variants in *RPE65* as the molecular cause of IRD in the studied cohort.

In 5 unresolved *USH2A* cases, reverse transcription PCR plus Oxford Nanopore sequencing characterized the splicing defects of deep intronic variants (c.8682-654C>G, c.9055+389G>A, and c.9959-2971C>T), a synonymous VUS (c.2139C>T), and a predicted loss-of-function duplication spanning an intron/exon junction (c.3812-3_3837dup).[43] These functional data enabled reclassification of VUS and established molecular diagnoses, demonstrating the value of LRS in resolving splice defects not readily interpretable by SRS alone.[43]

Long-read sequencing enabled detailed characterization of the splicing effects of *PHYH*:c.678+5G>T variant,[40] as well as of 3 splice variants in *PDSS1* (c.468-25A>G, c.722-2A>G) and *COQ5* (c.682-7T>G).[32] Li et al[44] used the PacBio single-molecule real-time sequencing to resolve autosomal recessive bestrophinopathy in a Chinese family. Long-read sequencing enabled identification of biallelic *BEST1* variants, including a novel intronic variant, c.867+97G>A.[44] Maggi et al[41] investigated Nanopore LRS as a tool to characterize aberrant splicing effects of IRD-associated variants. Nineteen variants from 15 IRD genes were analyzed.[41] Nanopore sequencing enabled precise quantification of isoforms and confirmed aberrant splicing for 13 of the 19 variants.[41] Functional evidence was incorporated into American College of Medical Genetics and Genomics reassessment, leading to upgraded classifications for several variants.[41] Additionally, LRS detected a deep intronic *USH2A* variant (c.4885+740A>T) in 2 siblings (homozygous) and in a sporadic case (heterozygous with a multiexon duplication).[47] In the study of Capasso et al,[48] long-read cDNA sequencing enabled the functional validation and reclassification of 2 previously identified *NMNAT1* 5' UTR splice-site VUS (c.-57+1del and c.-57G>A).

Inherited retinal dystrophies are genetically heterogeneous and often involve complex or hidden variants that challenge conventional SRS.[57] Advanced LRS technologies now offer more comprehensive genomic resolution, enabling accurate detection of difficult variants and supporting more informed clinical decision-making and patient management.

Resolving the precise configuration of complex SVs at base pair resolution is crucial for understanding their functional impact and pathogenic potential.[58] Traditional methods, such as PCR walking, are labor intensive, time-consuming and often unsuccessful, while WGS can be limited by high cost and mapping difficulties in low-complexity regions.[34] Long-read sequencing overcomes many of these barriers by capturing full-length pathogenic variants and enabling retrospective analysis of previously unresolved variants. McClinton et al[34] demonstrated this advantage in genes such as *CNGA1*, *CNGB1*, *EYS*, and *PRPF31*, where LRS improved diagnostic accuracy and clarified variant configuration. This capability can reduce diagnostic odysseys and facilitate earlier referral to appropriate clinical services.

Phasing is crucial for interpreting recessive IRDs, yet parental samples needed for segregation analysis are not always available.[49] A key advantage of LRS is its ability to determine parental haplotypes directly, addressing a significant challenge posed by SRS.[59] Nakamichi et al[20] demonstrated that targeted LRS of *USH2A* gene achieved high target enrichment, enabling haplotype reconstruction and phased variant identification in probands with Usher syndrome. Similarly, in a case where SRS was insufficient to resolve variant phase, ONT-based targeted LRS successfully identified the variants as being in trans using only the proband sample.[33] Consequently, LRS may provide a direct, interpretable, and clinically reliable strategy for variant phasing in IRDs.

In practice, these advantages translate to measurable clinical benefits: higher diagnostic yield, shorter time to diagnosis, and enhanced confidence in variant interpretation. Long-read sequencing is particularly valuable for patients who remain unsolved after standard gene panels or WES/WGS, those who suspected complex alleles, and for patients being evaluated for gene therapy eligibility where precise structural characterization is essential.

Challenges in applying precision medicine to IRDs may arise when genetic testing identifies VUS.[60] Long-read sequencing helps in reclassifying VUS in IRD cases by deciphering whether variants reside in cis or trans.[33] Phasing genetic variants is important for pinpointing those that could potentially be disease-causing.[33] By reclassifying VUS as benign or pathogenic, clinicians can make more informed decisions regarding patient management and treatment options.[33]

Inherited retinal dystrophies are in the spotlight of genetics-based therapies, including small molecule treatments, gene replacement, and stem cell approaches.[61] Long-read sequencing is a potent tool in the genetic diagnostic workup of *RPE65*-IRD cases.[56] Its ability to offer detailed and in-depth genetic data guarantees that patients receive accurate diagnoses, appropriate genetic counseling, and, where applicable, eligibility for gene therapy.[31] Gene-editing technologies such as CRISPR-Cas9 represent a major advance in IRD therapy, enabling precise correction of pathogenic variants.[62,63] The success of these therapeutic strategies depends on reliable identification and characterization of disease-causing variants, which is greatly enhanced by the higher resolution and accuracy of LRS. Moreover, LRS can be used to validate gene-editing outcomes by confirming the presence of intended modifications in gene-edited models.[64]

Despite remarkable progress, several challenges may limit the widespread adoption of LRS in clinical and research settings. One limitation of LRS is its reduced accuracy in detecting single nucleotide variants and small indel variants.[65] Although LRS can span complex genomic regions such as *RPGR*-ORF15, accurate resolution of these regions often requires manual review.[37] Additionally, repetitive and purine-rich regions can form secondary structures that may block the pores of MinION and Flongle flow cells of nanopore sequencing and increase cost due to the need for repeated reloading of sequencing libraries.[37] DNase I flow cell wash kit may solve this issue by unclogging and restoring the pores and therefore increasing read yields and depth at the ORF15 target locus.[37] Challenges such as limited yield, read accuracy, variable throughput, and high costs per base still hinder the routine use of LRS in high-throughput clinical settings and widespread adoption in large-scale projects.[66] However, leading LRS platform manufacturers have recently introduced technical innovations to address key limitations, substantially reducing error rates and overall performance improvements.[66,67] Assessing the cost-effectiveness of new sequencing technologies requires considering factors beyond reagent costs, such as labor and analysis time.[42] Nanopore enrichment sequencing, despite its higher initial cost per sample compared with NGS techniques like WES and WGS, offers significant time savings with only 6 hours for preparation and a day for sequencing, allowing for immediate variant analysis and interpretation of fewer variants, thus enhancing productivity and reducing overall costs.[42]

This review highlights the emerging role of LRS in advancing the genetic understanding of IRDs. While these findings highlight the potential of LRS to expand the genetic landscape of IRDs and contribute to solving aspects of missing heritability, the current evidence is largely limited to case reports and small series. Larger, systematic studies will be needed to confirm these preliminary observations and to clarify the clinical utility of LRS in routine diagnostic settings. As sequencing accuracy improves and costs decline, LRS is poised to become an integral component of genetic diagnostics, supporting precision medicine and the development of targeted therapies for IRDs.

Footnotes and Disclosures

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All authors have completed and submitted the ICMJE disclosures form.

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HUMAN SUBJECTS: No human subjects were included in this study. This study is a systematic review that analyzes data obtained from published, peer-reviewed literature that had received prior ethical approval and participant consent by the original investigators. No human or animal samples were collected. Therefore, the institutional review board of Beirut Arab University determined that approval and informed consent were not required. This review adhered to the principles of the Declaration of Helsinki and followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Checklist (Supplementary file 1).

The protocol was registered in the International Prospective Register of Systematic Review (CRD42024602173).

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Author Contributions:

Conception and design: Ibrahim, El Shamieh

Analysis and interpretation: Ibrahim, Chebly

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Data Availability: The data presented in this study are available upon request from the corresponding author.

Abbreviations and Acronyms:
BBS = Bardet-Biedl syndrome; **IRD** = inherited retinal dystrophy; **JBI** = Joanna Briggs Institute; **LRS** = long-read sequencing; **NGS** = next-generation sequencing; **ONT** = Oxford Nanopore Technologies; **PacBio** = Pacific Biosciences; **PCR** = polymerase chain reaction; **QUADAS-2** = Quality Assessment of Diagnostic Accuracy Studies (revised tool); **RoB** = Risk of Bias; **RP** = retinitis pigmentosa; **SRS** = short-read sequencing; **SV** = structural variant; **VUS** = variants of

uncertain significance; **WES** = whole-exome sequencing; **WGS** = whole-genome sequencing.

Keywords:
Inherited retinal dystrophies, Long-read sequencing, Next-generation sequencing, Variants, Missing heritability.

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